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# CORRELATION OF THE LIFE CYCLE OF A PARASITE WITH THE METAMORPHOSIS OF ITS HOST

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Apparently but few instances have been noted where a parasite changes in form *pari passu* with the changes in its host during metamorphosis. It would be interesting to know if such a correlation is a common one. Certainly further knowledge of such adaptations would assist in the identification of some parasites and in a better understanding of their life histories. The object of this paper is to describe what is apparently a clear instance of such a correlation between a gregarine parasite and its host.

## THE HOST AND METHODS OF HANDLING IT

The host of the gregarine under discussion is the fly, *Sciara coprophila,* a member of the family Mycetophilidae. It commonly occurs on house plants, feeding on their roots and breeding in the loose upper layers of the dirt in which the plants are grown. It may also be found under leaves and in the moist earth at the base of trees. The height of the breeding season is in April and early May, although it begins in March and continues at a slow rate all summer. The larva is about 6 mm. long at the maximum size, with a body color of cream or white and a black head. The pupa is half the length of the larva, and varies from deep cream to brown according to age. The adult female fly averages 3 mm. in length and the male, 2.5 mm. The duration of the larval existence is from eleven to twelve days; of the pupal, six days. The eggs hatch in about a week. All these figures fluctuate slightly with different conditions of food and temperature. The host has a complete metamorphosis, a matter which is of special significance in that it has an important bearing on the development of the parasite. In each of the three phases of metamorphosis there is accommodation for only a definite and limited phase of the parasite's cycle. For example, the trophozoite, or growing gregarine, is confined to the larva; the conjugating gregarine and the preliminary cyst formation, to the pupa; and the spore forming and sporozoite development, to the adult fly. The growing time of the parasite coincides with the feeding and growing time of the host; the preparation for reproduction

falls simultaneously with the period of sexual differentiation in the host; and the production of spores, the last step in the reproduction of the parasite, takes place in the adult fly which is itself ripe for the fertilization program.

So far as the writer knows such a correlation has not been thus far described for gregarines nor for any other parasite. Gregarines usually complete their entire cycle in the larval stage of the host, in case it is a host with complete metamorphosis or if they fail to form spores at the larval stage they may do so in the pupa or even in the adult. Léger (1892) found that certain gregarines hibernate with the host and continue development only when the host resumes activity. But in all these cases the element of chance, the chance of age operates; there is no regularity, no timed correlation of homologous phases of two animals as we have in the present instance.

All stages of the host were observed and with very few exceptions they showed heavy infections. Three lots have been used in this paper: Lot 1, collected by Mr. H. B. Hungerford on house plants, April 19, 1918; Lot 2, collected by the writer on a greenhouse snapdragon, May 16, 1920; Lot 3, collected by the writer on the soft earth at the base of trees growing on the campus of the University of Kansas, June, 1920. Several other species of Sciara, as well as some of the more distantly related members of the family Mycetophilidae, have been examined for gregarine infection, but none has been found thus far.

The infected larvae, pupae and flies were examined in some cases in smears immediately after capture. The freshly collected host was placed in a drop of normal salt solution. With a fine lancet the body was cut behind the jaws and with a needle the digestive tract and its accompanying glands were pulled out upon the slide. In the larvae the larger gregarines are visible through the intestinal walls and through the transparent cells of the ceca; and in the adult fly the dark spherical cysts show distinctly through the wall of the otherwise empty stomach. Infection, except at its inception, is therefore, easy to determine.

Other material was fixed before examination. Hot Schaudinn fluid was found best for preserving guts to be mounted whole, and both borax carmine and Mallory's chloride of iron hematoxylin were used for staining these mounts. Hot Bouin's fluid was employed for material to be sectioned. This material, which consisted of entire larvae, pupae and flies, was immersed two hours in the fixative, the chitinous body wall being first pierced with a needle in several places. Sections were cut 7 to 10$\mu$ thick and were stained by the Heidenhain iron-hematoxylin method as well as by Mallory's chloride of iron hematoxylin. Both gave satisfaction, but Mallory's stain was more often used because the process can be completed in half an hour and is superior for spore staining.

THE GREGARINE OF THE LARVAL HOST

When an infected larval intestine is drawn from the body upon a slide in normal salt solution, almost invariably large gregarines will be seen pushing through the tissue to free themselves. If the intestinal wall is broken by pressing the cover glass still other gregarines appear. These vary in size from very small to very large forms, but a glance will show them all to be in the trophozoite or growing stage.

By examining stained sections of the host of the same age the normal location of the parasites can be readily determined. The very young are in the cells which line the intestinal tract and the ceca (Fig. 1); and the older forms, the so-called sporonts, are free in the lumen of these organs. Thus we see that the parasite begins its development intracellularly. The time between the ingestion of the sporozoite and its entrance to the cell must be very short, because few free sporozoites are found in the intestine, while the very young intracellular trophozoites are numerous in the larvae. At its earliest intracellular stage (Fig. 2) the gregarine is not much larger than the nucleus of the cell which it has invaded, but by rapid growth it soon fills the cell and crowds the nucleus to one side (Figs. 3, 5, 6, 7). Intracellular development takes place impartially in the ceca and in the midgut. Usually both places are invaded. Likewise the sporonts occupy the lumen of both of these organs but wander into the intestine during the metamorphosis of the larva into the pupa.

From a smooth and very minute sphere measuring 10$\mu$ in diameter the parasite grows to fifteen or twenty times that diameter before bursting the cell membrane. Meanwhile it is developing a polycystid structure, which may be easily overlooked, because of the folded condition imposed by the narrow cell limits (Figs. 5, 6, 7, 8, 35). The epimerite is small and button-like and it is doubtful whether it is ever functional because it disappears before normal emergence of the parasite from the cell. Another structure is also lost to the gregarine about this time, the septum between the protomerite and the deutomerite. Although vestigial in character both the epimerite and the protomerite are a definite part of the early development of this gregarine and show its phylogenetic relationship to the polycystids.

By the time the sporont is free in the lumen it retains only a knob-like remnant of a protomerite and the epimerite has vanished, sometimes leaving a starlike scar to mark its point of attachment. The deutomerite of the full grown sporont is relatively long, narrow and spatulate, tapering sharply posteriorly. In it, about midway, lies the nucleus containing from one to three or more chromatin bodies. Occasionally, deeply stained granules are scattered through the cytoplasm. Sporonts often attain a length of 200$\mu$ in the intestinal cavity. Such forms usually lie close against the wall but are not attached to

it (Fig. 34). The head end is toward the head of the host unless there are large numbers of parasites, in which case the gregarines swing the head end toward the intestinal wall. The sporonts are always solitary.

That many separate infections have taken place is indicated by the wide variation in the development of the parasites. Some are in the youngest intracellular stage while others are apparently mature sporonts; but, not withstanding this, no time is gained in the larval host. All sporonts have met a closed door and must postpone further development until the host passes over into the pupal phase. No larva, at any age, was found to harbor parasites later than the sporont stage.

### THE GREGARINE OF THE PUPA

There seems to be a period of quiescence in the gregarine's development at the end of the larval phase, for we find the intestine of the late larva crowded with sporonts of various size and very few intracellular forms. This pause is preparatory to the next phase, pseudo-conjugation, which in most gregarines proceeds in the larva. But in the present case the process of development stops short in the larva and recommences apparently under the stimulus of the pupal stage of the host.

A change of position comes with the pupal phase. The sporonts which have lain with their anterior end toward the anterior end of the host begin to writhe about with a snakelike movement. This motion is visible through the gut wall and after some time accomplishes the union of the sporonts in pairs. Previous to this joining so great an elongation of the sporonts has taken place that they are hardly recognizable as the same animal. They have also become narrow except at the anterior end, which has widened perceptibly. The union is head to head with usually no dimorphism of conjugants. Occasionally one sees a ball and socket union, such as described by Duke (1910), and sometimes a pair consists of one short and one long conjugant, but these variations are not constant enough to be of any significance. Figs. 12 to 17 show the various stages of pseudo-conjugation which occur in the pupa.

A shortening of the copula next follows, resulting in a bilobed sphere. There seems to be a condensation of the entire mass at this time, though it proceeds most rapidly at the anterior ends bringing the nuclei of the two animals close together. The posterior tip is the last to lose itself in the rounded mass and remains for a time protruding like a tail. The pupa of the mid phase shows a varied picture (Fig. 14). The copulae are in every stage from late contraction to early segmentation of the bispherical cysts.

Sometimes before the cyst is yet rounded the nucleus of each half has begun to divide. This continues until the cytoplasm is well dotted

with chromatin patches, and is itself condensing around them to form the so-called pearl stage of the gregarine life history. Unfortunately, material was not available for a detailed study of the processes immediately following this, and the union of the gametes is therefore undetermined. All divisions are plainly mitotic (Fig. 21).

The sphere early begins a revolving movement which is connected with the formation of the cyst wall. This wall makes its appearance first as a granular secretion around the sphere (Fig. 20). There is some doubt about its composition. In some of the sections it has a distinctly cellular look after it is well along in development, but the initial structureless secretion together with the reports of such authors as Léger and Duboscque (1909) and Siedlecki (1900) makes the gelatinous theory of structure more probable. In the older cysts the wall is stratified, deeply pigmented and opaque.

### THE GREGARINE OF THE FLY

Perhaps the most noteworthy changes that the parasite undergoes in the fly is the formation of the spores. These have begun at the time of the pearl stage in the pupa, but little more has been accomplished there than the division of the sphere into minute bodies. It is possible that these bodies are gametes and unite for the production of the spore, as with *Monocystis agilis* and many others. But conjugation was not observed and I am inclined to believe that the spores are not zygotes. The spore wall appears first irregular in outline and very thin, but as the spores move further apart the wall thickens, assumes a bi-conical form and at last develops a winged edge along one side (Fig. 31). Division of the nucleus begins about this time and continues until in the oldest flies the spores contain frequently eight patches of chromatin.

None of these changes can be seen in the early fly except in sections because the spores are all contained in cysts. These cysts are often so numerous in the stomach of the host that they distort that organ (Figs. 23, 24). But later the cyst walls disintegrate and the spores are loosened. There are no ducts for the passage of these spores and there is no definite break; the wall simply crumbles away from the contents. For a time the boat-shaped bodies within hang together but at last they become so widely distributed that in an old fly there is no region of the digestive tract from esophagus to rectum which does not harbor the loose spores (Figs. 25 and 26).

No further development of the spore was observed in the host, but when cysts are placed in normal salt solution the sporozoites become free within a few hours. The spores were not isolated and the exact number of sporozoites to the spore was not determined. Our assumption of eight rests upon the eight divisions of the spore chromatin as

previously described. Infection of the new host is probably through the ingestion of spores and not of cysts, since the cysts have broken down before the natural death of the host.

## CLASSIFICATION AND AFFINITIES

The genus Schneideria, in which I have placed the gregarine of this paper, was created by Léger in 1892 and contains up to the present only three species: *S. mucronata, S. coronata* and *S. praecox*. I have found no description of *S. praecox*.

The characteristics of the genus as Léger gives them are:

1. Epimerite in the form of a thick, horizontal dish with milled border.
2. Sporonts, solitary.
3. Sporonts with a single segment; i. e., no protomerite in the adult stage.

The present gregarine possesses the second and third characteristics, but the epimerite is too rudimentary to be described as a thick milled disk. It is merely a button-like elevation of but short duration. This difference may justify the creation of a new genus, but if so, I shall leave it to the systematists.

That the specimen is neither *S. mucronata* nor *S. coronata* I have shown by the following table. It is without doubt a new species which I have called *S. metamorphosa* because of the close correlation of its life cycle with the metamorphosis of its host.

| | *S. mucronata* | *S. coronata* | *S. metamorphosa* |
|---|---|---|---|
| Host | *Bibio marci* | *Sciara nitidicollis* | *Sciara coprophila* |
| Development | May complete entire developed cycle in larva | May complete entire developed cycle in larva | Requires all three phases: larva, pupa and imago for completion of cycle |
| Location in host | Limited to cells of ceca until time for encystment, only then passing into intestine | Limited to cells of ceca until time for encystment, only then passing into intestine | Not limited to ceca for early developed phases, but using both ceca and intestine |
| Epimerite | Epimerite with milled border and a short style at center, functions for a short time after sporont emerges from cell | Epimerite more sessile than in *S. mucronata*, a milled border and possessing no style, functions for a short time after sporont emerges from cell | Epimerite a mere bud, plain border and no style, disappears while gregarine is still in cell, never functions at any time |
| Form of Cyst | Bi-spherical | Unknown | Spherical |
| Maximum size | 800 $\mu$ | 1,000 $\mu$ | 300 $\mu$ |

Léger found *S. mucronata* and *S. coronata* to be true dicystids during their intracellular existence and for a short time after emergence from the cell. The epimerite is functional for a while before it is shed, leaving the sporont a monocystid in form. This cycle he found slightly modified in the genera Gametocystis and Sphaerocystis. In these the

epimerite is more ephemeral than in the above cases, disappearing completely by the time the intracellular growth is attained. There is no trace of the dicystid nature at the time of emergence.

*Schneideria metamorphosa* conforms to both Gametocystis and Sphaerocystis as regards the ephemeral epimerite, but during the intracellular phase it possesses besides this an equally distinct and ephemeral protomerite. For a brief time the gregarine is a true polycystid. This is more evidence for Léger's statement that "The true dicystids are united to the polycystids by insensible gradations."

Each of the three phases of the host's development is linked with a definite and limited phase of the parasite's development. And a still closer view shows this correlation to be physiologic as well as morphologic: the parasite and host feed and grow at the same time; have their quiescent periods at the same time; and carry on the propagation program simultaneously. The writer suspects that this extraordinary adaptation of the parasite's life cycle to fit that of its host may be frequent in the gregarine group and may explain the incompleteness of so many gregarine life histories.

## SUMMARY AND CONCLUSIONS

In the fly, *Sciara coprophila,* a new host has been found for a gregarine previously noted only in *S. nitidicollis.*

It is the fourth species recorded in the rare genus Schneideria Léger, and the only one found outside of France.

It differs from the other recorded species in that (*a*) its intracellular phase is polycystid; (*b*) the epimerite is never functional; and (*c*) both epimerite and protomerite are shed before gregarine leaves the cell.

Its life cycle presents a remarkable correlation with that of its host; it begins as an intracellular parasite in the midgut of the larva of the fly, *Sciara coprophila.* Here it undergoes a polycystid development, possessing an epimerite, a protomerite and a deutomerite. While still inside the cell all divisions atrophy except the deutomerite, and the parasite emerges a monocystid. In the larva the gregarine goes no farther than the sporont stage. In the pupa, sporonts, solitary up to this time, unite in twos, head to head, shorten into spheres and begin segmentation, meanwhile laying down a cyst wall. In the adult fly the formation of the spores is completed and the spore chromatin divides preliminary to the production of the sporozoites.

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## Explanation of Plates

All drawings were made with camera lucida except Fig. 23, which is a free hand drawing.

## Explanation of Plate XVIII

All figures from larva of *Sciara coprophila*

Fig. 1.—Median longitudinal section of larva of *Sciara coprophila* showing gregarines in all states of growth. Some occupy the cells of the intestinal and cecal walls and others are free in the lumen of the intestine.

Figs. 2, 3 and 4.—Sporozoite just before and after entering the cells of the intestine.

Figs. 5, 6 and 7.—Early intracellular stages of the parasite undergoing the polycystid phase. The protomerite and deutomerite are at first nearly equal in size. Note that the epimerite is functionless.

Fig. 8.—A late intracellular phase. The deutomerite is gaining length over the protomerite.

Fig. 9.—Small gregarine from the cecal lumen; very unusual remnant of epimerite which is ordinarily shed or absorbed inside the host cell.

Fig. 10.—Enlarged section of mid gut showing various stages of development of the parasite.

Fig. 11.—One of the large free sporonts measuring over 200 $\mu$ long. Note slight constriction at anterior end and lack of septum. On the anterior end is the scar of the lost epimerite.

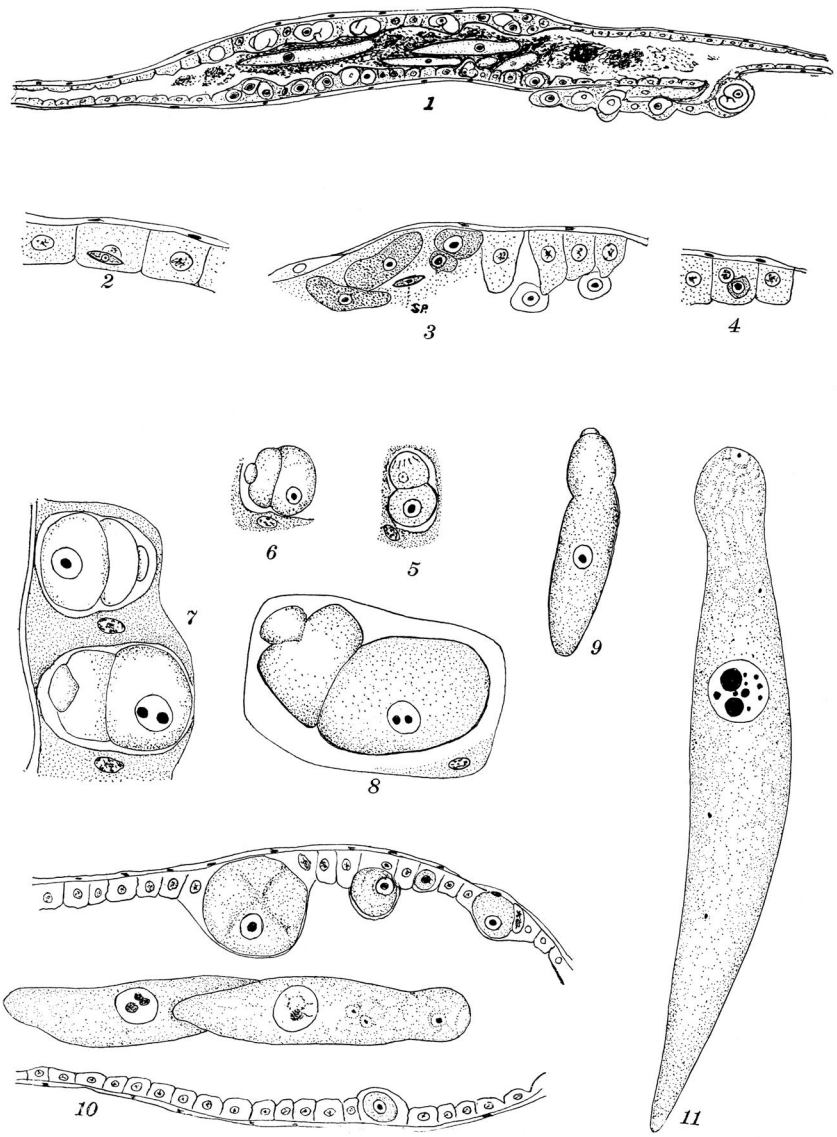



PLATE XVIII

EXPLANATION OF PLATE XIX

All figures from pupa of *Sciara coprophila*.

Fig. 12.—Free hand sketch of stomach of late pupa showing gregarines in pseudo-conjugation bursting through wall after accidental puncture.

Fig. 13.—Median longitudinal section of early pupal mid gut showing copulae just getting together.

Fig. 14.—Stage later than Figure 13; contraction of copulae has begun; some are in segmentation stage.

Fig. 15.—Pair of conjugants extended.

Fig. 16.—Beginning of contraction of conjugants.

Fig. 17.—Continuation of contraction of conjugants.

Figs. 18 and 19.—The early sphere.

Fig. 20.—Early segmentation of sphere, and beginning of cyst wall.

Fig. 21.—Drawn under oil immersion to show mitosis; cyst wall in process of formation.

Fig. 22.—Enlarged view to show method of union of two conjugants.

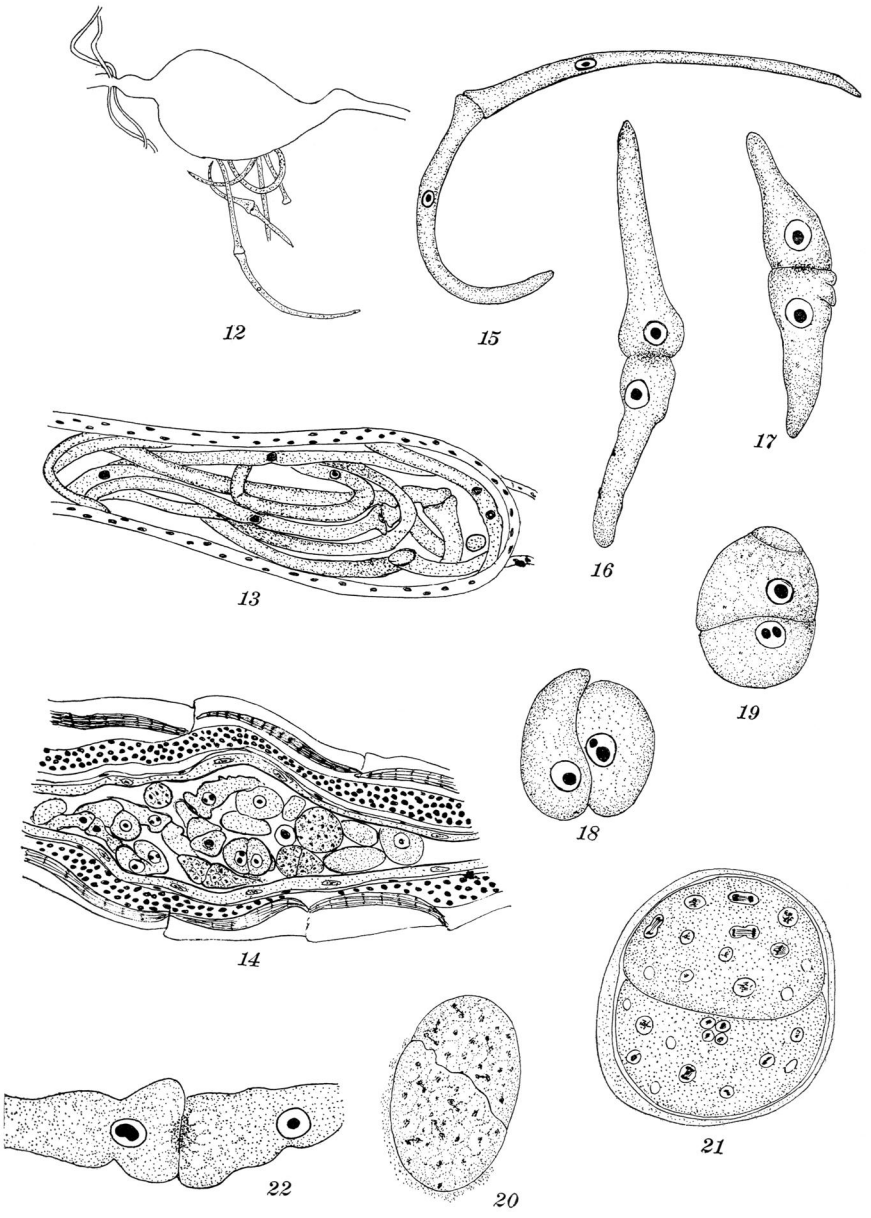



PLATE XIX

EXPLANATION OF PLATE XX

All drawings from the adult fly.

Fig. 23.—Free hand drawing of fly stomach showing cysts through body wall.

Fig. 24.—Section of fly stomach showing cysts and otherwise empty lumen.

Fig. 25.—Stomach and intestine of fly showing breaking up of cysts and dissemination of spores even to rectum.

Fig. 26.—Fly stomach showing cysts completely disintegrated with spores widely scattered. Even esophagus of this specimen showed loose spores. Absence of food in the digestive tract shows that these spores date from larval infection.

Fig. 27.—Early cyst showing irregular spores.

Fig. 28.—Spores loosening from each other and cyst wall breaking away.

Fig. 29.—Boat-shaped spores fully formed and loose inside inner cyst wall.

Fig. 30.—An enlarged view of the uninucleate spores.

Fig. 31.—Division of nucleus preliminary to sporozoite formation.

Fig. 32.—A free spore.

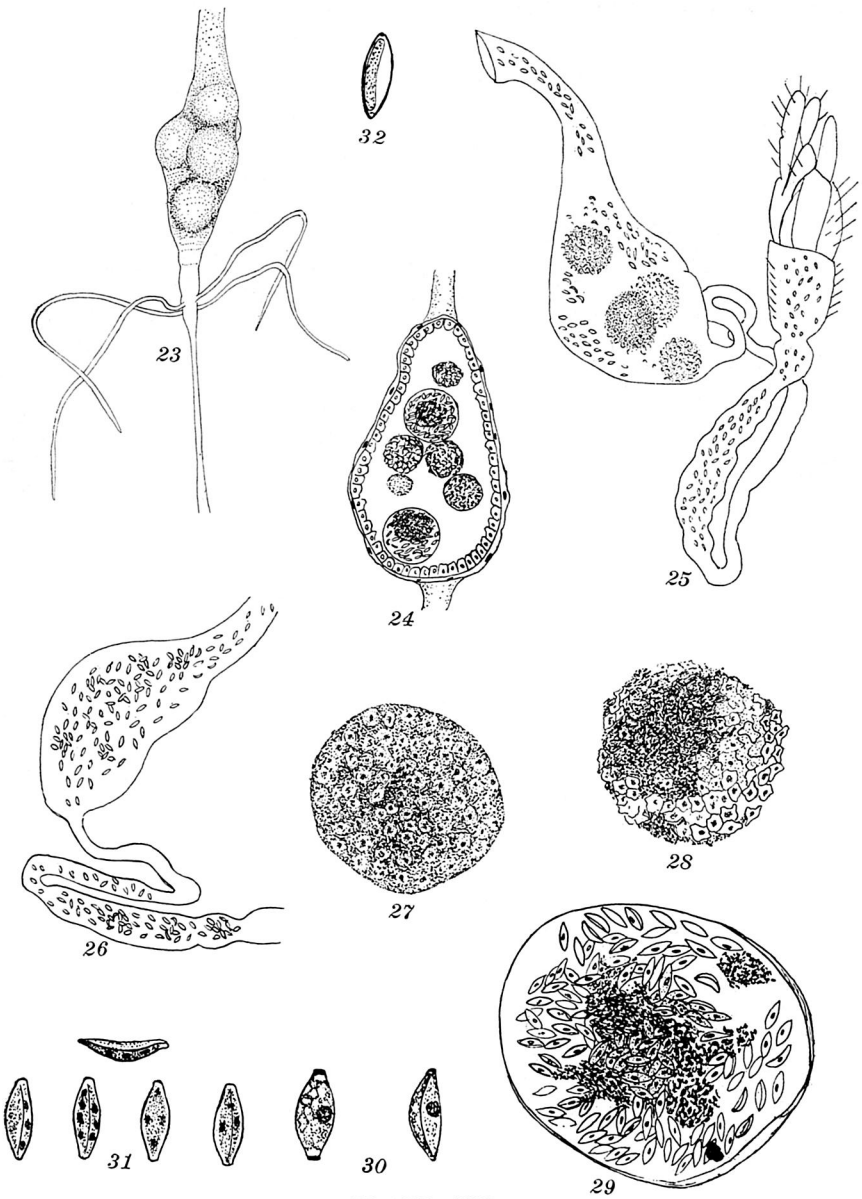



PLATE XX

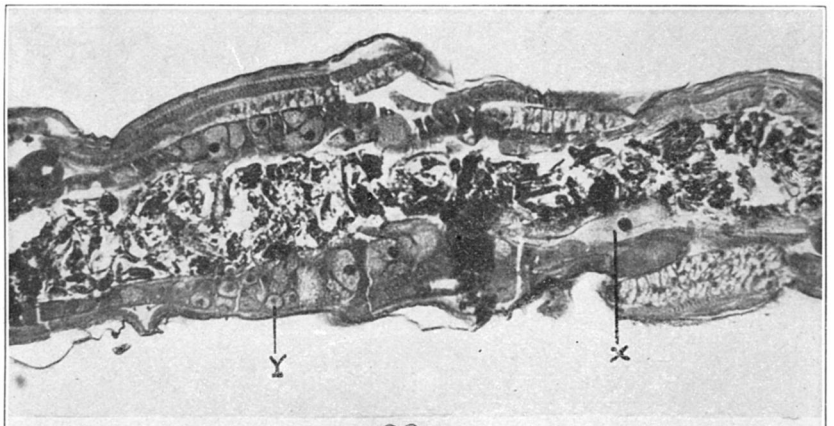
## Explanation of Plate XXI

Photomicrographs of median longitudinal sections of larva.

Fig. 33.—Intracellular development of gregarine in intestinal wall of larval host. At x a sporont is lying free in the lumen. × 160.

Fig. 34.—Typical adult sporont with tapering posterior and knob-like anterior end, lying in intestinal lumen. × 160.

Fig. 35.—Highly magnified trophozoite (y) still in intestinal cell. It shows the polycystid form through which *Schneideria metamorphosa* passes in its intracellular growth stage. The protomerite is distinct. The epimerite is the button which fits wedge-like into its free end, and is somewhat more difficult to distinguish. × 350.

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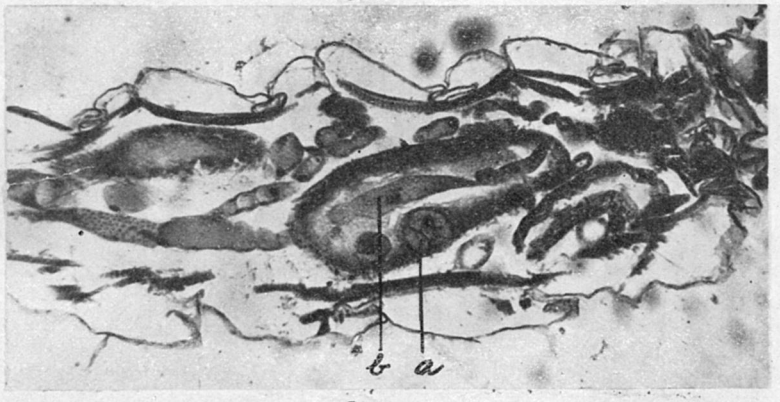


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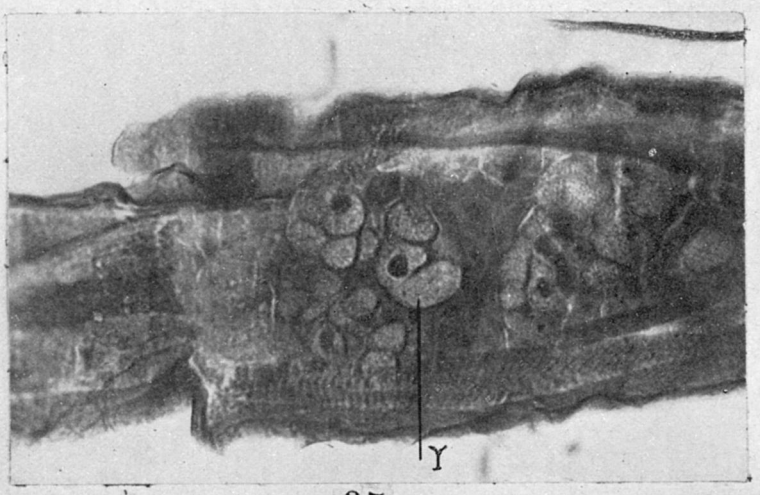


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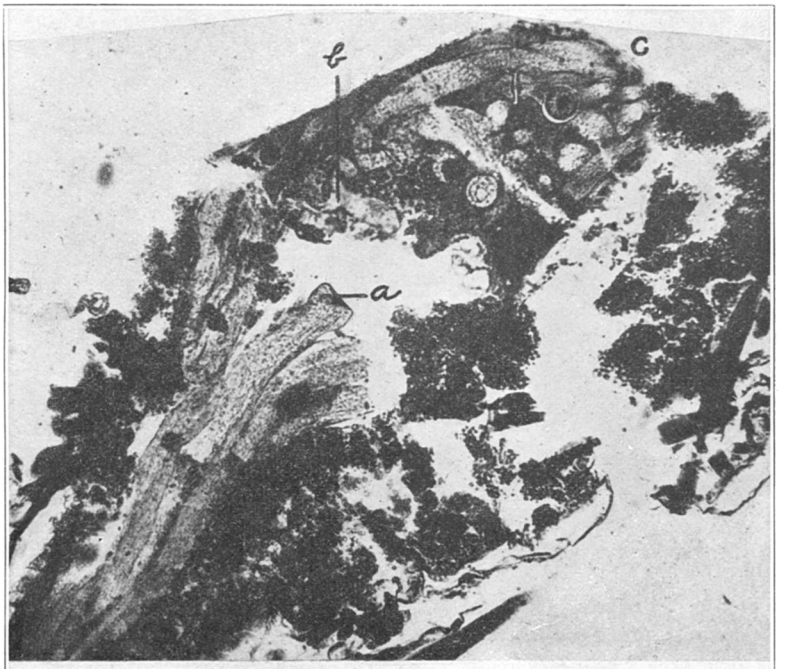
PLATE XXI

EXPLANATION OF PLATE XXII

Photographs of median longitudinal sections of pupa.

Fig. 36.—Pseudo-conjugation. At *a* is a sporont transformed for pseudo-conjugation. It is greatly elongated and has a flattened head end. At *b* and *c* can be seen the heads of the two copulae united. This is a very large larva, and the gregarines are proportionally large. × 160.

Fig. 37.—Beginning of cyst formation. At *a* the pair of conjugants has not quite formed the sphere. At *b* this is accomplished. At *c* the large nucleus of each half has divided into numerous parts. × 160.

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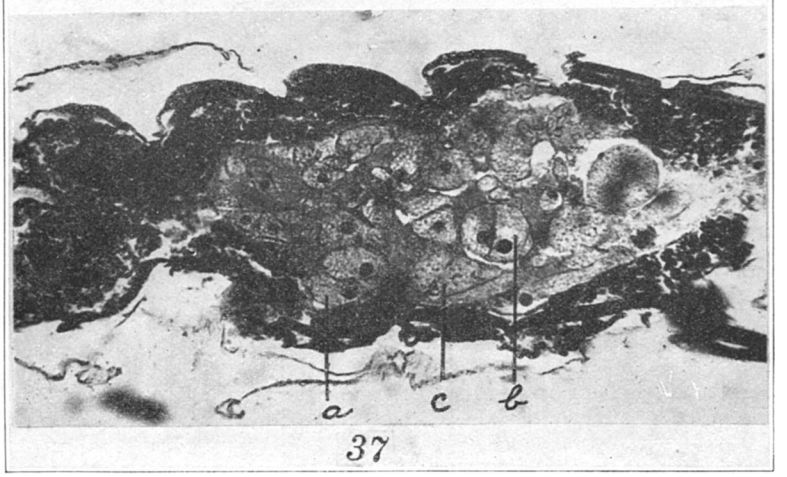


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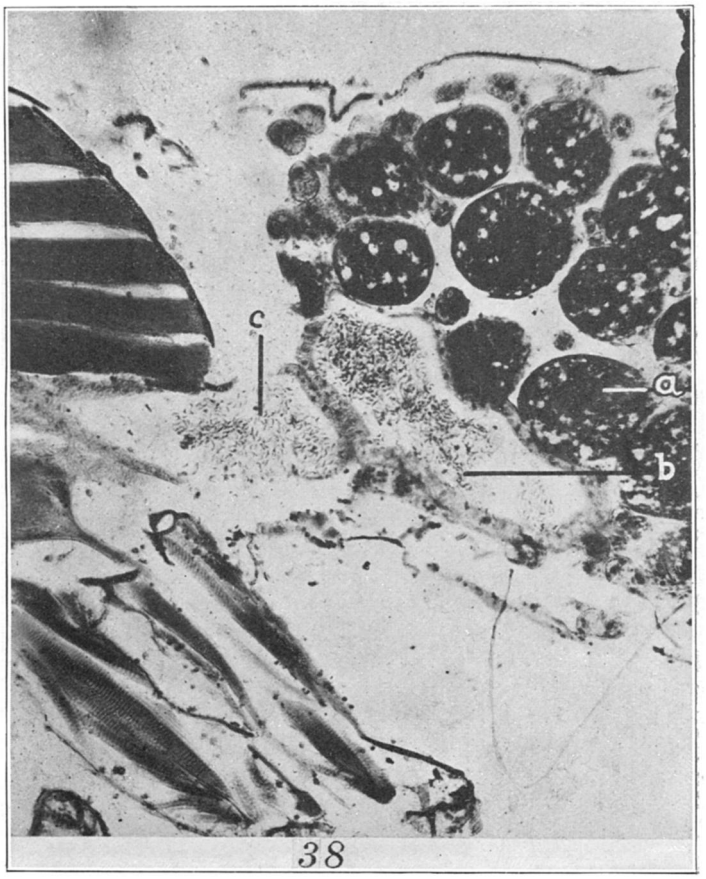
PLATE XXII

EXPLANATION OF PLATE XXIII

Fig. 38.—Median longitudinal section of mature female fly with abdomen filled with eggs (*a*). Stomach (*b*) and esophagus (*c*) are filled with spores already free from cyst. × 160.

Fig. 39.—Copulating pair of *Schneideria metamorphosa* showing heads (*a*). × 160.

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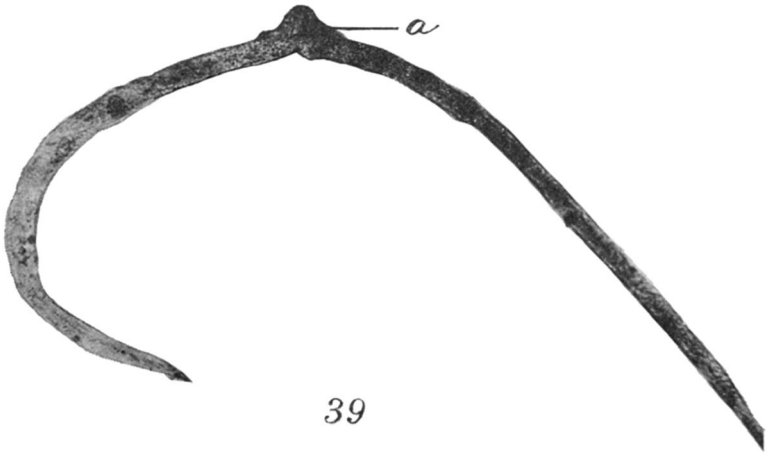


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PLATE XXIII